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MONOCYTES IN HEALTH AND DISEASE – MINIREVIEW

K. R. Karlmark[1,*], F. Tacke[2] and I. R. Dunay[3]

[1]Deparment of Dermatology and Allergology, Philipps University, Marburg, Germany
[2]Department of Internal Medicine III, University Hospital Aachen, Aachen, Germany
[3]Institute of Medical Microbiology, Otto-von-Guericke University, Magdeburg, Germany

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Monocytes are important cell types of the innate immune system. Recent scientific evidence suggests that monocytes not only play a crucial role in our innate immune system by defending the host from intruding microbial pathogens but they also contribute to the pathogenesis and progression of diseases such as liver fibrosis, atherosclerosis, multiple sclerosis, and tumor metastasis. In addition, monocytes and monocyte-derived macrophages play a crucial beneficial role in the liver fibrosis regression, muscle regeneration, and the clearance of the β-amyloid plaques in Alzheimer's disease. Here, we summarize the origin, plasticity, and pathogenic potential of monocytes and monocyte-derived macrophages, as well as their positive role in the regression of some common diseases. Elucidating the comprehensive immunological role of monocytes will provide therapeutic advantages in either controlling disease progression or favoring the regression of the disease state.

Keywords: monocytes, chemokine receptor, infection and injury, inflammation, tumor metastasis, liver fibrosis, neurodegeneration

Monocyte facts

i) Monocytes consist of two principle subsets (Mouse: Gr-1^{hi} (Ly6C^{hi}) and Gr-1^{low} (Ly6C^{low}); Human: CD14^{++}CD16^{-} and CD14^{+}CD16^{+}).
ii) Gr-1^{hi} subsets employ CCR2 to emigrate from the bone marrow.
iii) Monocytes differentiate into macrophages and/or dendritic cells and thus modulate innate and adaptive immunity.
iv) Recent evidence suggests that monocytes play a crucial role in defending the host against infections, but may also promote diseases, such as liver fibrosis, atherosclerosis, multiple sclerosis, and tumor metastasis.
v) Monocytes and monocyte-derived macrophages also promote disease regression such as fibrosis regression, muscle regeneration after injury and degradation of amyloid fibrils in Alzheimer's disease.

Introduction

Monocytes are indispensable leukocyte subsets of the innate immune system that play a major role in defending the host from invading pathogens. In the 1880s, Elie Metchnikoff demonstrated the phagocytic capacity of these blood derived cells, until recently the knowledge of their origin and fate remained elusive. Two different monocyte subsets have now been identified based on their chemokine receptors expression and migration patterns, which have distinct functions in both innate and adaptive immune responses *(Table 1)*. Moreover, these subsets also can give rise to distinct dendritic cell subsets that in turn control T and B cell responses further. Based on recent scientific data, our current knowledge of these monocyte subsets has expanded immensely and allowed us to understand their critical contributions in the pathogenesis of liver fibrosis, atherosclerosis, Alzheimer's disease, autoimmune diseases like multiple sclerosis and interestingly in tumorigenesis. Furthermore, like a double-edged sword, monocytes, and monocyte-derived macrophages also a play a role in reducing the severity of diseases, such as fibrosis and Alzheimer's disease by degrading or clearing up collagen-I deposits and β-amyloid fibrils, respectively. In addition, monocytes induce muscle regeneration after injury by producing TGF-β as well as play a crucial role in the control of microbial infections.

Monocyte origin, homeostasis, and plasticity

Among the blood leukocytes, the mononuclear phagocytic cells or monocytes represent a subgroup of cells with a plasticity to develop into macrophages or dendritic cells. Human and mouse monocyte subsets are more complex than currently recognized; however, a current report offer

*Corresponding author: Karlin Raja Karlmark PhD; Department of Dermatology and Allergology, Philipps University-Marburg, Baldingerstrasse D-35033 Marburg, Germany; Phone: +49-6421-58-61406; Fax: +49-6421-28-65729;
E-mail: karlmark@med.uni-marburg.de

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Table 1. Monocyte associate chemokines and chemokine receptors

Monocyte subset	Chemokine receptors	Corresponding ligands
Classical monocytes	CCR1	MCP-2, MIP-1α (CCL3), RANTES (CCL5)
Mouse: Gr-1^{hi}	CCR2	MCP-1 (CCL2), MCP-3 (CCL7), MCP-5
Human: CD14^{++}CD16^{-}	CXCR2	CXCL1 (GROα), CXCL2 (GROβ), CXCL3 (GROγ)
Non-classical monocytes	CX_3CR1	CX3CL1 (Fractalkine)
Mouse: Gr-1^{low}	CCR5	CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES)
Human: CD14^{+}CD16^{+}	CCR6	CCL20 (MIP-3α)

a great comparison between the subsets in the two species [1]. It has been shown that mammalian monocytes consist of two major subsets based on their chemokine receptor expression which may play different roles during homeostasis and inflammation [2]. In humans, they are CD14^{++}CD16^{-} CCR2^{+}CX_3CR1^{low} and CD14^{+}CD16^{+} CCR2^{-}CX_3CR1^{+}, whereas in mouse they represent Gr-1^{hi} and Gr-1^{low}, respectively. Moreover, they migrate differentially based on their chemokine receptor expression [2–5] *(Table 1)*. Previous data have revealed that monocytes require the chemokine receptor CCR2 to egress from the bone marrow [5, 6]. It has been recently shown that circulating TLR-ligands can induce production of MCP-1, the major monocyte chemoattractant by the bone marrow mesenchymal and progenitor cells, which in turns binds to its major receptor CCR2 expressed by the Gr-1^{hi} monocytes and promotes their egress from the bone marrow into the circulation [7] (*Fig. 1*). The precise origin, conversion, and biological function of Gr-1^{low} monocyte subsets still remain elusive. In addition to the bone marrow, it has been shown that the spleen contains a huge reservoir of monocytes under normal homeostatic conditions. Upon injury or inflammation, splenic monocytes increase their motility, exit spleen, and migrate toward the site of injury or inflammation [8].

Regarding their plasticity, monocytes migrate to peripheral tissues during parasitic infections and play an essential role in the host defense [5, 9, 10]. Upon emigration from blood, the monocytes can also differentiate into TNF-α, iNOS-producing dendritic cells (so-called TipDCs) and control the microbial load, reported in different bacterial and fungal infections [11–13]. Under inflammatory conditions of the skin, Gr-1^{hi} monocytes migrate into the epidermis and differentiate into Langerhans cells (LC) [14] and within the lung, Gr-1^{hi} CCR2^{+} monocytes give rise to CD103^{+} pulmonary dendritic cells [15]. In the experimental model of liver fibrosis, we have shown that during prolonged hepatic injury, Gr-1^{hi}CCR2^{+} monocytes migrate to the injured area and differentiate into either iNOS (classically activated) or Arginase (alternatively activated) producing macrophages, based on the Th1/Th2 environment, respectively, and modulate the disease progression [4].

Functions

The individual monocyte subsets that have been illustrated *(Figs 1 and 2)* were defined based on their functional properties. During homeostatic conditions, mononuclear phagocytes in circulation represent 5–12% of the total leukocyte population. Within this population, the subsets contribute approximately to equal percentage of the total monocyte population in mice [2, 4, 6]. During onset of infection with various pathogens or upon inflammation, the subset ratio changes dramatically, and it shifts toward the Gr-1^{hi} CCR2^{+} subset, which initiates the effector function and pro-inflammatory role by producing IL-6, TNF-α, and iNOS [4–6, 10, 13, 16]. In humans, the non-classical subset CD14^{+}CD16^{+} has been found to play a proinflammatory role. The so-called TipDCs are the direct descendents of the Gr-1^{hi} CCR2^{+} monocytes [11, 15, 17] and favor the clearance of the ongoing infection *(Fig. 1)*. In the experimental models of liver injury [4, 18], autoimmunity to the CNS [19], lung injury [20], and different parasitic infections [5, 9, 10], the Gr-1^{hi} CCR2^{+} monocytes are the immediate responders to the inflammation.

The functions of Gr-1^{low} monocytes are less clear. Some experimental data indicate that Gr-1^{low} CX_3CR1 monocytes might be a more anti-inflammatory subset and may suppress inflammation by producing IL-10. Perhaps, they require their major chemokine receptor CX_3CR1 for their own survival rather than for the recruitment [18, 21] to the site of infection or inflammation. These subsets gave rise to CD103^{-} pulmonary dendritic cells under experimental conditions [15].

Associated pathologies

Infection and injury

Monocytes are crucial for an effective immune response to most pathogens. They are equipped with chemokine receptors and adhesion receptors that modulate migration from the blood to the site of infection or injury. Previously, it has been suggested that monocytes use the chemokine receptor CCR2 for their recruitment to infected tissues. However, recent data suggest that monocytes, especially the classical Gr-1^{hi} subset, use CCR2 for their egress from the bone marrow into the blood circulation [5, 6]. Moreover, during infection, the circulating TLR-ligands can induce the synthesis of MCP-1, the major binding partner for CCR2, by the bone marrow mesenchymal stem and progenitor cells [7] this enables the monocytes to exit and migrate toward the site of infection *(Fig. 1)*. Although neutrophils are the immediate responders to any infection and injury (within few hours), monocytes are the key players in modulating the inflammation by producing cytokines, such as IL-1β, IL-6, TNF-α, as

well as iNOS, and thus controlling the early phase of different infections [5, 9, 10, 22, 23] or injury [24]. Recent studies using CCR2 $^{(-/-)}$ mice or highly specific depletion strategies confirmed that monocytes, but not neutrophils, are important mediators in the control infections [9, 22]. Furthermore, during certain microbial infection the Gr-1^{hi} CCR2^{+} monocytes can differentiate into TipDCs and produce TNF-α, iNOS [11, 17, 25, 26] and favor the clearance of the ongoing infection *(Fig. 1)* and may promote an adaptive immune response by presenting the antigens to lymphocytes.

In the experimental model of muscle injury, monocytes migrate to the site of injury and facilitate the clearance of the apoptotic cells [24]. Interestingly, after engulfing the apoptotic bodies, the monocytes switch phenotypes from pro-inflammatory to anti-inflammatory and begin to produce IL-10 and TGF-β1, thus facilitating the regeneration of the injured muscle *(Fig. 2)*.

Liver fibrosis

Prolonged challenge to the liver such as HBV, HCV infection, or chronic alcoholism leads to deposition of type-I collagen to the extracellular space as a healing mechanism, which finally impairs the normal function of the liver. Clinically, this stage is called liver fibrosis. In the experimental models of liver fibrosis, Gr-1^{hi} CCR2^{+} monocytes migrate rapidly into the liver [4] and accelerate the fibrosis by producing pro-inflammatory and pro-fibrogenic cytokines, such as IL-6, TGF-β1, and thus activating the major collagen producing cells in the liver *(Fig. 1),* the hepatic stellate cells. Our experimental studies using the CCR2 knockout mice showed a reduced liver fibrosis in ccr2-deficient animals due to a reduced infiltration of Gr-1^{hi} monocytes into the injured liver [4]. Similar studies with the CX_3CR1-knockout mice showed a more severe liver fibrosis by massive infiltration of Gr-1^{hi} CCR2^{+} monocytes [18]. These different studies clearly showed the negative role of infiltrating Gr-1^{hi} CCR2^{+} monocytes in fibrosis disease progression. Nevertheless, infiltrated monocytes can also differentiate into macrophages and produce matrix metalloproteinases MMPs *(Fig. 2)*. These MMPs degrade the collagen-I deposit and facilitate the fibrosis regression, in case the challenge to the liver is stopped. Under these conditions, monocytes act like a double-edged sword either facilitating the severity of the disease or at the

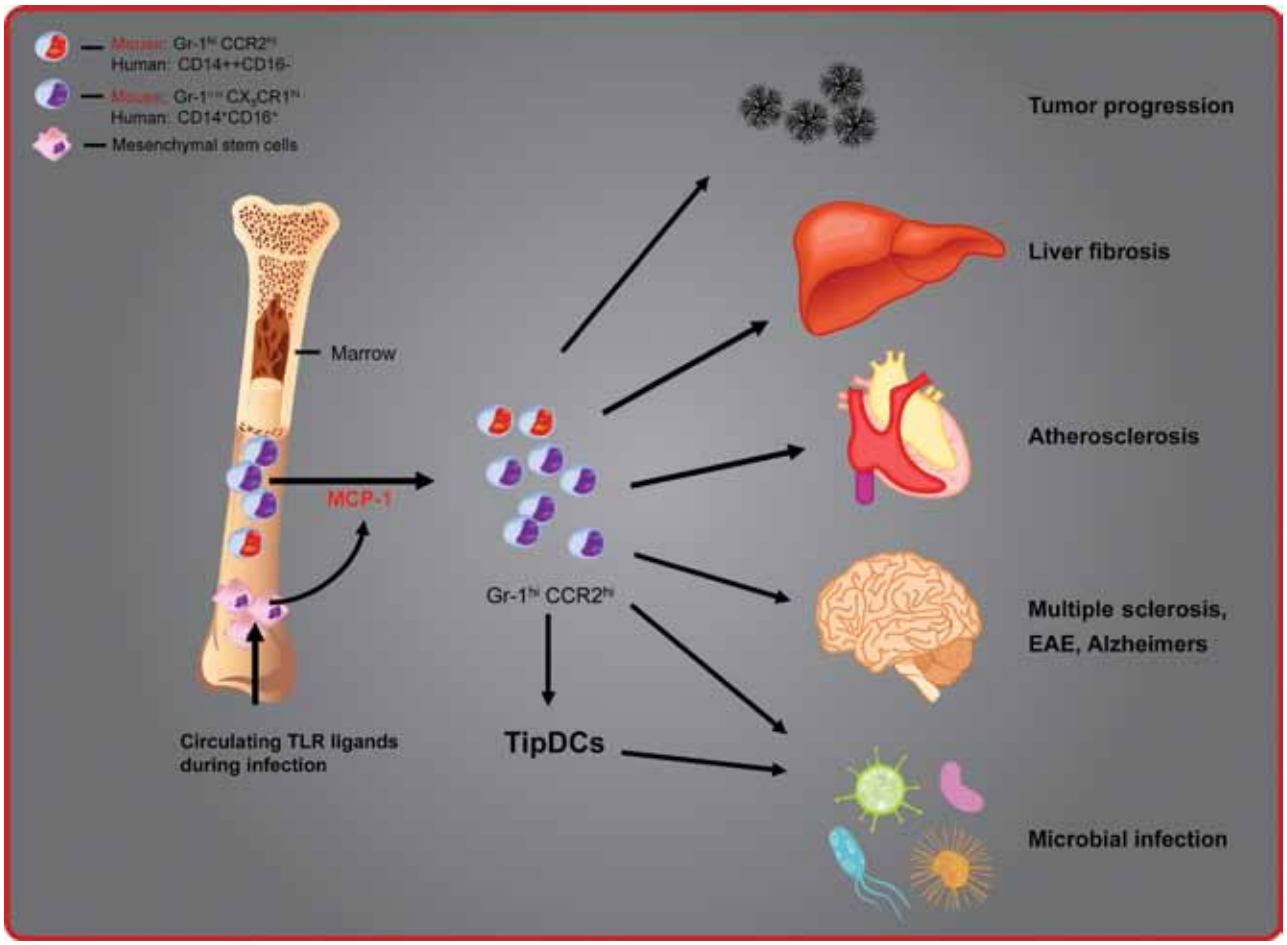

Fig. 1. Monocytes in the mammalian blood circulation consist of two major subsets based on their chemokine receptor expression, migration and functional properties. Gr-1^{hi} (Ly6C^{hi}) monocyte subset egression from the bone marrow is CCR2 dependent. Monocytes are also circulating precursors of non-lymphoid dendritic cells. Recent scientific evidence suggests that monocytes not only play a crucial role in our innate immune system by defending the host from intruding microbial pathogens, but they also contribute to the pathogenesis and progression of diseases such as liver fibrosis, atherosclerosis, multiple sclerosis and tumour metastasis

later stage facilitates the regression of the disease. Interestingly, a recent finding showed that under Th2-dependent inflammatory conditions, tissue macrophages accumulate by self-renewal [27] rather than recruitment from the blood. This distinct mechanism during Th2 inflammatory conditions contradicts the conventional hypothesis of macrophage recruitment from the blood circulation.

Atherosclerosis

Thickening of the arterial wall by cholesterol accumulation or so-called atherosclerosis is one of the leading killers in the modern world. Among the other aetiologies, chronic inflammation to the arteries, which leads to the accumulation of macrophages (foam cells) and their defective clearance of the LDL proteins, is the major cause of atherosclerosis. In the experimental model of atherosclerosis, such as ApoE-deficient mice, the Gr-1^{hi} monocytes accumulate in the atherosclerotic plaques by using the chemokine receptors CCR2, CCR5, and CX_3CR1 [3] and play a major role in plaque progression *(Fig. 1)*. Recent data revealed that in the experimental model of atherosclerosis, $CX_3CR1^{-/-}$ mice had a reduced severity of disease due to the lower survival rate of infiltrating monocytes/macrophages [28], thus modulating atherosclerosis progression.

Multiple sclerosis, EAE, and Alzheimer's disease

It has been shown that infiltrating Gr-1^{hi} CCR2^{+} mononuclear phagocytes with morphological similarities to endogenous microglial cells can enter the adult brain [16, 27]. In addition, chimeric CCR2$^{-/-}$ GFP mice revealed reduced microglial numbers under experimental conditions [16]. Furthermore, during experimental induction of autoimmune encephalomyelitis by immunizing with MOG_{35-55} peptides, Gr-1^{hi} CCR2^{+} monocytes rapidly migrate into the brain as early effector cells and increase the severity of the disease phenotype [19], suggesting a role of these monocytes during the early phase of CNS inflammation and autoimmunity. Monocytes isolated from patients undergoing type-I interferon treatment (IFN-β) showed reduced production of the pro-inflammatory cytokine IL-1β [30].

Monocytes and microglia function is still being controversially discussed in the context of beta-amyloid removal in Alzheimer's disease (AD), with beneficial or detrimental effects due to cytokine release and the phagocytosis

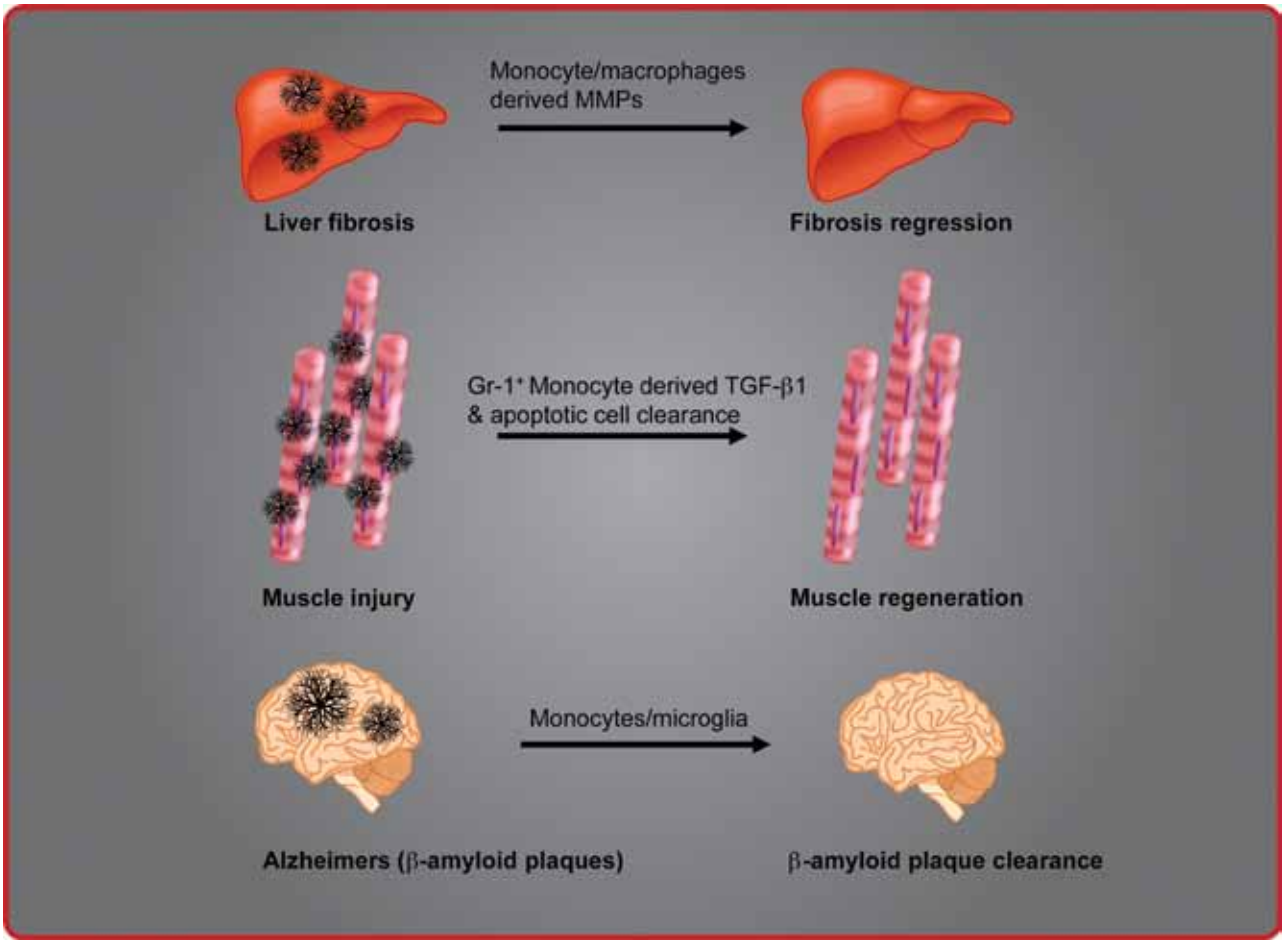

Fig. 2. Monocytes as a therapeutic target. Monocytes and monocyte-derived cells play a role in reducing the severity of diseases such as fibrosis and Alzheimer's disease by degrading or clearing up collagen-I deposits and β-amyloid fibrils, respectively. In addition, monocytes induce muscle regeneration after injury by producing TGF-β

function [16, 31–33]. Most of these analyses were done at single time points in different animal models. However, recent experimental data in long-term experiments show that microglia are active in the removal of amyloid during early stages of plaque formation in AD [32]. This functional activation is limited by a still unknown mechanism and leads to a 'hibernating' microglia species enclosed the growing amyloid deposits. The phagocytosis function is not only modulated by amyloid-beta but also by specific mitochondrial activity changes [34] that show a direct relation of cerebral ATP levels and microglia activity. A current report demonstrated that CCR2 deficiency in the transgenic mouse model of Alzheimer's disease provokes a rapid cognitive impairment closely correlated with brain amyloid pathology [35]. On the positive side, monocytes genetically engineered to produce the protease called 'neprilysin' arrest the amyloid deposition in an experimental mouse model, suggesting a beneficial therapeutic role of these cells *(Fig. 2)* in treating Alzheimer's disease.

Tumor progression

Monocytes play pleiotropic functions in many inflammation-induced pathologies. Novel data show that monocytes play a critical role in carcinogenesis as well as tumor metastasis. It has been known that monocyte-derived myeloid suppressor cells can suppress the anti-tumor activity of T-cells by producing IL-10. In an experimental model of chemical-induced carcinogenesis, histidine carboxylase (HDC)-deficient mice showed a severe tumor progressive phenotype [36], accompanied by a massive influx of $CD11b^{+}Gr\text{-}1^{+}$ monocytes and their impairment in maturation. An independent study revealed that vascular endothelial growth factor (VEGF) produced by the infiltrating $Gr\text{-}1^{+}$ monocytes in mammary cancer promotes tumor metastasis [37] into the lung. By blocking CCL2 (MCP-1), the major monocyte chemoattractant, the authors showed that the ability of the tumors to form metastases was reduced [37]. These findings indicate a crucial role for these $CD11b^{+}Gr\text{-}1^{+}CCR2^{+}$ monocytes in carcinogenesis. Very recently, it has been shown that tissue factor (TF) expressed by tumor cells correlates with the infiltration of monocytes/macrophages, tumor cell survival and metastasis. Pharmacological inhibition of tissue factor-mediated coagulation abrogated monocyte/macrophage recruitment and tumor cell survival in an experimental model of lung mestastasis [38].

Summary

In this review, we have highlighted the recent findings pertaining to the important role of monocytes and monocyte-derived cells in various immunological and pathological scenarios: like responding and defending from infections, in addition to promoting diseases such as liver fibrosis, atherosclerosis, multiple sclerosis, Alzheimer's, and tumour metastasis. Nevertheless, we should take into account the beneficial roles played by these immune cells in every disease such as fibrosis regression, clearance of β-amyloid plaques in Alzheimer's disease, and defending against microbial invasions. Many scientific studies from around the world independently revealed that monocytes act as a double-edged sword, either beneficial or detrimental. Finally, these cells are an indispensable part of our innate immune system, and they make an excellent target for effective therapeutic intervention in the fight against several human diseases.

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